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► **To cite this version:**

Henrik Zetterberg, Kaj Blennow, Eric Hanse. Amyloid β and APP as biomarkers for Alzheimer's disease. *Experimental Gerontology*, 2009, 45 (1), pp.23. 10.1016/j.exger.2009.08.002 . hal-00547640

HAL Id: hal-00547640

<https://hal.science/hal-00547640>

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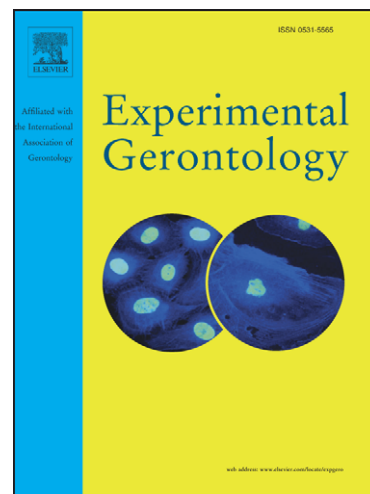
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PII: S0531-5565(09)00159-4
DOI: [10.1016/j.exger.2009.08.002](https://doi.org/10.1016/j.exger.2009.08.002)
Reference: EXG 8633

To appear in: *Experimental Gerontology*

Received Date: 26 May 2009
Revised Date: 7 August 2009
Accepted Date: 13 August 2009



Please cite this article as: Zetterberg, H., Blennow, K., Hanse, E., Amyloid β and APP as biomarkers for Alzheimer's disease, *Experimental Gerontology* (2009), doi: [10.1016/j.exger.2009.08.002](https://doi.org/10.1016/j.exger.2009.08.002)

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Amyloid β and APP as biomarkers for Alzheimer's disease

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Abstract

1
2 Intense research during the past decade has aimed at dissecting the molecular pathogenesis of
3
4 Alzheimer's disease (AD). Primarily, the focus has been directed towards brain amyloid
5
6 pathology and its relation to synaptic and neuronal loss. Clearly, AD is associated with
7
8 accumulation of amyloid β ($A\beta$) in the brain. Further, the results of many experimental
9
10 studies suggest that certain forms of $A\beta$ may act as initiators in the disease process with
11
12 potent toxic effects at the synaptic level. Molecular aberrations in the AD brain are reflected
13
14 in the cerebrospinal fluid (CSF). Core CSF biomarkers include secreted $A\beta$ and amyloid
15
16 precursor protein (APP) isoforms, $A\beta$ oligomers and β -site APP-cleaving enzyme 1 (BACE1).
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18 This article reviews recent research advances on CSF and plasma $A\beta$ -related biomarkers for
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20 AD and how they may reflect pathogenic changes in AD-affected neuronal networks. We also
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22 consider their usefulness in clinical practice and in clinical trials.
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32 Keywords: cerebrospinal fluid, biomarkers, Alzheimer's disease, amyloid, long-term
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34 potentiation
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1. Cardinal features of Alzheimer pathology

1 Pathological hallmarks of Alzheimer's disease (AD) include synaptic and neuronal loss,
2
3 astrocytosis, intraneuronal inclusions of hyperphosphorylated tau protein in neurofibrillary
4
5 tangles and extracellular deposits of amyloid β ($A\beta$) protein in senile plaques. These lesions
6
7 are typically located in the areas of the brain that are involved in memory consolidation,
8
9 especially the limbic cortex including the hippocampus. Although sometimes seen also in
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11 aged non-demented individuals, a skewed $A\beta$ homeostasis that promotes the accumulation of
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$A\beta$ in the brain is believed by many to represent the earliest event in the Alzheimer disease process (Blennow et al., 2006).

$A\beta$ is generated from amyloid precursor protein (APP), a membrane-spanning protein with one transmembrane domain, by enzymatic digestion involving β - and γ -secretase activities (Fig. 1). Many studies have identified the presenilins, along with nicastrin, anterior pharynx defective-1 and presenilin enhancer-2, as the functional components of the γ -secretase complex (De Strooper, 2003). The molecular composition of γ -secretase is conserved across metazoans (Campbell et al., 2006; Francis et al., 2002; Zetterberg et al., 2006), and it serves many important functions by acting as the primary protease needed for Notch signaling as well as the processing of many other transmembrane receptors (Selkoe, 2001). In 1999, several research groups published evidence demonstrating that a significant part of the β -secretase activity originates from an integral membrane aspartyl protease encoded by the β -site APP-cleaving enzyme 1 (*BACE1*) gene (Hussain et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999). Other substrates for BACE1 include the sialyltransferase ST6Gal I (Kitazume et al., 2003; Kitazume et al., 2001), the adhesion protein P-selectin glycoprotein ligand-1 (PSGL-1) (Lichtenthaler et al., 2003), β subunits of voltage-gated sodium channels (Wong et al., 2005), APP-like proteins (APLPs) (Li and Sudhof, 2004), $A\beta$ itself (Fluhrer et

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2 al., 2003) and neuregulin 1 (NRG1), an axonally expressed factor required for
3 oligodendrocyte differentiation and myelination (Hu et al., 2006; Willem et al., 2006).
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6 7 **2. Alzheimer's disease causation – what is the evidence for amyloid β as a central** 8 9 **molecule in the disease process?** 10

11
12 Apart from the firm association of amyloid pathology in the brain with clinical symptoms of
13 AD and the salient amyloid-promoting effect of known mutations causing familial AD
14 (Blennow et al., 2006), there are numerous data showing strong biological effects of certain
15 forms of A β , especially at the synaptic level. These effects will be reviewed below, as they
16 provide further support for A β as a core neurochemical marker of AD.
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27 Three main synaptotoxic effects of A β have been recognized; inhibition of long-term
28 potentiation (LTP), removal of synaptic glutamate receptors and elimination of glutamate
29 synapses (Fig. 2). Glutamate synapses constitute 85-90% of the synapses in the mammalian
30 cortex (De Felipe et al., 1997; Megias et al., 2001), and their plasticity, i.e. their ability to
31 adapt their efficacy in response to specific patterns of neuronal activity, is thought to be the
32 basis for learning and memory (Kessels and Malinow, 2009; Neves et al., 2008). The
33 postsynaptic membrane of the glutamate synapse is typically equipped with ionotropic AMPA
34 and NMDA receptors. The AMPA receptors are responsible for the normal, fast electrical
35 signaling whereas the NMDA receptors, which are highly permeable for calcium, are required
36 for the induction of LTP and its counterpart long-term depression (LTD) at these synapses.
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LTP, a lasting increase in synaptic efficacy, typically involves an expansion of the synapse with more AMPA receptors, whereas the opposite is typical for LTD (Kessels and Malinow, 2009; Neves et al., 2008).

1 The inhibitory action of A β on NMDA receptor-dependent LTP has been shown in different
2 experimental settings, including genetic modifications leading to overproduction of A β
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4 (Chapman et al., 1999; Kamenetz et al., 2003), synthetic A β (Cullen et al., 1997; Lambert et
5
6 al., 1998; Raymond et al., 2003), cell-produced A β (Walsh et al., 2002), A β oligomers in
7
8 human cerebrospinal fluid (CSF) (Klyubin et al., 2008) and A β oligomers from AD brains
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10 (Shankar et al., 2008). Although much remains to be learned about the nature of the A β
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12 species that exert this LTP-inhibiting effect, it seems related to soluble oligomers of the
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14 peptide (Townsend et al., 2006; Walsh et al., 2005). In any event, aggregated forms of A β in
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16 fibrils and plaques appear not to affect synaptic function (Shankar et al., 2008), which might
17
18 be the reason for why some individuals are cognitively normal in spite of high amounts of
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20 fibrillar A β in their brains (Reiman et al., 2009). Extended clinical follow-up is, however,
21
22 needed before it can be concluded whether these individuals indeed are protected from A β
23
24 toxicity by effective sequestration of A β in inert aggregates or by other factors that neutralize
25
26 the toxicity of A β , or if they are bound to deteriorate cognitively when followed over many
27
28 years. It is also possible that some individuals can compensate for the effect of brain
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30 pathology as posited by the cognitive reserve concept (Rolstad et al., 2009).
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33 It is interesting that the LTP-inhibiting effect of A β oligomers is fast, within tens of minutes
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35 after the application, indicating receptor-mediated effects. The receptor for soluble A β
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37 oligomers remains however elusive. It has been shown that A β binds to glutamatergic, but not
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39 to GABAergic, synapses, in close vicinity of NMDA receptors (Lacor et al., 2007). It has also
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41 been shown that A β oligomers downregulate insulin receptors (De Felice et al., 2009).
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44 Further, insulin can antagonize the LTP-inhibiting effect of A β (Lee et al., 2009; Townsend et
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46 al., 2007). Recently, the prion protein was identified as a strong binding partner for A β
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1 oligomers (Lauren et al., 2009). In any case, A β -mediated inhibition of LTP in the medial
2 temporal lobe, including the hippocampus, in animal models satisfactorily explains the
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4 hallmark symptom of early AD, namely anterograde amnesia of declarative learning.
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7 However, although synaptic degeneration with loss of synaptic terminals is a characteristic
8
9 feature of the disease (Davidsson and Blennow, 1998), it is still unknown whether LTP is
10
11 defective in patients with AD. Further, a recent clinical trial on A β immunization showed that
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13 high anti-A β titers were accompanied by clearance of amyloid from the brain but did not
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15 prevent progressive neurodegeneration or improve cognition or survival (Holmes et al., 2008).
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17 Thus, further studies are needed on the relation between A β oligomers and neurodegeneration
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21 in AD.
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27 In contrast to LTP, LTD appears not inhibited by A β . On the contrary, A β has been shown to
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29 promote and mimic certain forms of LTD by removing glutamate receptors from the synapse
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31 (Cheng et al., 2009). There have been mixed results regarding the extent to which AMPA
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33 and/or NMDA receptors are removed from the synapse (Chang et al., 2006; Kamenetz et al.,
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35 2003; Snyder et al., 2005; Ting et al., 2007). It is however interesting that, in studies showing
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37 removal of AMPA receptors, there seems to be a total removal of all AMPARs in a subset of
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39 synapses, rather than a reduction of the number in most synapses (Kamenetz et al., 2003; Ting
40
41 et al., 2007). This phenomenon is akin to AMPA silencing, a form of synaptic plasticity that is
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43 otherwise restricted to the developmental period and that may be a premonition for synaptic
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45 elimination (Abrahamsson et al., 2007; Groc and Choquet, 2006; Xiao et al., 2004).
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54 A decreased density of glutamate synapses is a characteristic hallmark of AD that has been
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56 replicated in several animal models of AD (Almeida et al., 2005; Hsia et al., 1999; Koffie et
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58 al., 2009; Terry et al., 1991; Ting et al., 2007). As indicated above, it is possible that the
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1 removal of glutamate receptors is a prelude for the elimination of the synapse. For example,
2 AMPA receptors bind to, among many other proteins, pentraxins in the synapse and the
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4 binding site of pentraxins for AMPARs is also a binding site for the complement factor C1q
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6 (Perry and O'Connor, 2008). The classical complement cascade has been shown to be
7
8 critically involved in synapse elimination during brain development (Stevens et al., 2007) and
9
10 in the pathogenesis of AD (Fonseca et al., 2004). Thus, removal of synaptic AMPARs may
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12 leave the synapse exposed to complement attack and subsequent physical elimination by
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14 microglia.
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22 During brain development there is a tug-of-war between constructive (e.g. synaptogenesis)
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24 and destructive (e.g. synapse elimination) processes that in concert sculpt the growing
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26 nervous system (Waites et al., 2005). Recent studies suggest that A β and its precursor protein
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28 APP may have important physiological roles promoting destructive processes during brain
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30 development. Thus, they appear to be involved in eliminating synapses (Priller et al., 2006),
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32 pruning neuritis (Nikolaev et al., 2009) and restricting mature forms of LTP (Townsend et al.,
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34 2006). These functions are possibly related to a large production of A β early in development,
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36 and, as a consequence, maybe also formation of soluble A β oligomers. Although information
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38 about the developmental profile of the concentrations of A β in the brain is lacking, the
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40 prerequisites for a large production seem to be there. The peak expression of APP and the
41
42 enzymes necessary for the production of A β , γ -secretase and BACE1, coincides with the
43
44 period of most intense synaptogenesis and synaptic elimination in the rodent hippocampus
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46 (Chiocco and Lamb, 2007; Kirazov et al., 2001; Moreno-Flores et al., 1999). In the normal
47
48 adult brain there is presumably a low balanced level of constructive and destructive processes
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50 and the production of soluble A β oligomers is also kept low. In the AD brain destructive
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52 processes accelerate while constructive processes remain at a low level, leading to neuronal
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1 degeneration. Thus, based on these similarities between the putative effects of A β
2 developmentally and pathophysiologically, it can be hypothesized that the pathogenesis of
3 AD involves reactivation, or “retrogenesis”, of the destructive mechanisms involved in the
4 development of the brain (Wasling et al., 2009).
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10 11 **3. A β -related proteins as core neurochemical markers of AD**

12 As reviewed above and illustrated in figure 1, A β peptides are formed by proteolytic cleavage
13 of the transmembrane protein APP by BACE1 and γ -secretase (the amyloidogenic pathway)
14 which results in shedding of an extracellular soluble N-terminal fragment of APP, termed
15 sAPP- β , along with A β . However, APP may undergo at least two and most likely several
16 different processing pathways (Andreasson et al., 2007). In the non-amyloidogenic pathway,
17 APP is cleaved at the α -site in the middle of the A β domain. This cleavage obliterates A β
18 formation, instead yielding an N-terminal sAPP- α fragment, which is found extracellularly
19 and in the CSF. The remaining C-terminal APP stub is further digested by the transmembrane
20 protein complex γ -secretase into small peptides involved in intracellular signaling pathways.
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40 Levels of sAPP- α and sAPP- β are unaltered (Olsson et al., 2003; Zetterberg et al., 2008), or
41 mildly elevated (Lewczuk et al., 2008) in sporadic AD, and correlate tightly with each other
42 (Lewczuk et al., 2008; Zetterberg et al., 2008). Although BACE1 is a membrane-bound
43 protein, an extracellular isoform produced by membrane-shedding is found in CSF, and CSF
44 BACE1 activity is increased in AD patients (Holsinger et al., 2006; Holsinger et al., 2004;
45 Verheijen et al., 2006), as well as in subjects with mild cognitive impairment (MCI) and
46 prodromal AD, as compared to controls (Zetterberg et al., 2008; Zhong et al., 2007). The
47 overlap between cases and controls with regards to CSF BACE1 activity is, however, too
48 large to allow for individual disease risk predictions.
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2 The 42 residues long A β isoform (A β 42) is highly hydrophobic and forms oligomers and
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The 42 residues long A β isoform (A β 42) is highly hydrophobic and forms oligomers and fibrils that accumulate in extracellular plaques (Andreasen and Zetterberg, 2008). This process is most likely facilitated by the E4 isoform of apolipoprotein E (apoE) encoded by the AD-associated *APOE* ϵ 4 allele (Prince et al., 2004; Reiman et al., 2009). Deposition of the peptide in plaques is considered the underlying basis for the approximately 50% decrease in CSF A β 42 levels seen in AD (Andreasen and Zetterberg, 2008). Although not entirely undisputed, this theory is supported by the strong correlation between low CSF A β 42 levels and high numbers of plaques in the neocortex and hippocampus (Strozyk et al., 2003), as well as high retention of the amyloid-binding positron emission tomography (PET) tracer Pittsburgh Compound-B (PIB) in the brain (Fagan et al., 2006; Forsberg et al., 2007).

However, since some neurodegenerative diseases present with low CSF A β 42 levels in the absence of plaques, other reasons for the reduced CSF A β 42 levels in AD cannot be ruled out. Recently, it was found that multiple sclerosis goes with reduced CSF A β 42, which is probably caused by upstream downregulation of BACE1 and APP expression secondary to neuroinflammation (Mattsson et al., 2009a).

Since CSF levels of the shorter A β 40 isoform are unchanged or increased in AD, it has been proposed that measurement of the A β 42/A β 40 ratio might be superior to A β 42 alone (Fukuyama et al., 2000; Kanai et al., 1998; Mehta et al., 2000; Schoonenboom et al., 2005; Shoji et al., 1998), even in early stage disease (Hansson et al., 2007). It is now well established that CSF A β 42 or CSF A β 42/A β 40 in conjunction with total tau (T-tau) and phospho-tau (P-tau) that reflect the axonal degeneration in AD (Ballatore et al., 2007) identify and predict AD with sensitivity and specificity figures of 80-95% (Hampel et al., 2004;

Hansson et al., 2006; Herukka et al., 2005; Mattsson et al., 2009b; Zetterberg et al., 2003).

Table 1 shows typical biomarker changes in different disease groups.

The discovery of shorter A β isoforms in brain and CSF has made it clear that A β constitutes a large family of peptides with considerable length variations (Portelius et al., 2008). The shorter peptides likely result from the ability of BACE1 and γ -secretase to execute catalytic cleavages at different positions in APP, as well as the probable involvement of other APP- and A β -degrading proteases. There are also numerous secreted APP/A β peptides that span the β -secretase site, which complicates the picture further (Portelius et al., 2009). More studies are needed to determine the clinical value of CSF measurements of detailed A β peptide patterns, as compared to CSF A β 42 alone.

Whereas all human beings produce A β , not all get synaptic impairment and AD. One explanation for this enigma would be that only specific forms of A β , not expressed in the general population, cause AD. The preeminent candidate to date is soluble A β oligomers that seem the most synaptotoxic but reliable methods for measuring A β oligomers in biological fluids are needed to determine the validity of this hypothesis. Promising studies using unconventional methods that have been difficult to standardize suggest that A β oligomers can be measured in CSF in the femtomolar range (Georganopoulou et al., 2005; Pitschke et al., 1998). However, the low concentration presents a methodological challenge and highlights the need for method development.

Recently, a method based on stable isotope labeling and mass spectrometric analysis of immunocaptured A β was presented (Bateman et al., 2006). This technique allows for determination of production and clearance rates of total A β in CSF and plasma and may help

1 addressing the fundamental question if patients who develop AD have increased production or
2 decreased clearance of the peptide. Its value as a diagnostic tool is unknown, but recent data
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4 show that it is very sensitive to identify biochemical effects of secretase inhibition in human
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6 subjects (Bateman et al., 2009).
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11 While it is clear that A β 42 along with tau protein levels in CSF are useful in clinical routine
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13 (Andreasen et al., 2001; Frisoni et al., 2009) and probably also to detect biochemical effects
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15 on amyloid deposition and axonal degeneration of novel drug candidates against AD
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17 (Bateman et al., 2009; Kadir et al., 2008; Lannfelt et al., 2008), it has been difficult to find
18
19 reliable amyloid biomarkers in peripheral blood (Irizarry, 2004; Zetterberg, 2008). Many
20
21 studies have examined plasma levels of A β in the context of sporadic AD but the findings are
22
23 contradictory. Some groups report high, others low A β 42 concentration or A β 42/A β 40 ratio
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25 as a risk factor for AD (Irizarry, 2004). In addition, longitudinal studies indicate that plasma
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27 A β levels may change during the course of the disease (Mayeux et al., 2003). Finally, and
28
29 perhaps most importantly, there is no correlation between the levels of A β 42 in plasma and
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31 CSF (Mehta et al., 2001; Vanderstichele et al., 2000) or between plasma concentrations of
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33 A β 42 and A β 40 and A β accumulation in the brains of patients with AD (Fagan et al., 2006;
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35 Freeman et al., 2007).
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46 **4. Limitations and pitfalls**

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48 A key weakness with regards to CSF biomarkers for AD today is the lack of external control
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50 programs that allow for harmonization of biomarker data between laboratories. There is
51
52 considerable inter-laboratory variation, especially with regards to A β 42 measurements also
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54 when the same kit is used (Buerger et al., 2009; Verwey et al., 2009). Efforts are currently
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56 ongoing to unveil the factors underlying the variation and to establish a global quality control
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1 program for the best established biomarkers, T-tau, P-tau₁₈₁ and A β 42. There is also variation
2 between centers with regards to the clinical characteristics of included subjects in studies
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4 (Mattsson et al., 2009b). These differences affect optimal cut-off points to distinguish AD
5 from controls and other disease groups. Altogether, it is at this stage advised against using
6 published reference or cut-off values without making sure that the new measurements are
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8 harmonized against the published data.
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16 Finally, it is very important never to interpret biomarker data in isolation but in the context of
17 the whole clinical picture. The positive predictive value for CSF T-tau, P-tau and A β 42 is
18 very good (80-95%) in MCI patients but low (<30%) in unselected populations due to the
19 powerful influence of disease prevalence on test performance (Mattsson and Zetterberg,
20 2009). Further, CSF biomarkers may be positive 5-10 years before clinical presentation of the
21 disease (Gustafson et al., 2007; Ringman et al., 2008), making it important to consider and
22 rule out other illnesses, e.g., depression, as contributing to the cognitive symptoms also in
23 biomarker-positive patients.
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39 **5. Conclusions and future research directions**

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41 Intense research has led to the development of CSF biomarkers reflecting different aspects of
42 AD pathogenesis. Currently, validated and reliable biomarkers exist for amyloid pathology
43 and Alzheimer-type axonal degeneration. The biomarkers are clinically useful and may serve
44 as inclusion criteria in clinical trials of anti-AD drug candidates. Ongoing anti-amyloid drug
45 development programs will demonstrate the possible utility of amyloid-related biomarkers in
46 dose-finding and proof-of-mechanism studies. Reliable methods to measure A β oligomers
47 that may be specifically related to AD onset and progression would be a valuable tool in AD
48 diagnostics.
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Acknowledgments

We wish to acknowledge the support of the Swedish Research Council (grants 2006-6227, 2006-3505 and 2006-2740), Alzheimer's Association (award number NIRG-08-90356), the Royal Swedish Academy of Sciences, cNEUPRO and the Swedish Brain Power project.

Conflicts of interest

The authors declare no conflicts of interest.

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Legends

1
2 **Fig. 1.** Schematic representation of amyloidogenic APP-processing. In brain, three major
3
4 isoforms (APP₇₇₀, APP₇₅₁ and APP₆₉₅) exist due to differential splicing of exon 7 that contains
5
6 the Kunitz protease inhibitor domain (KPI) and exon 8 that contains the OX2 antigen domain.
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8 BACE1 cleaves APP after residue 671, which causes the secretion of sAPP- β and the
9
10 retention of a 99 residue C-terminal fragment (CTF). This fragment can undergo further
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12 cleavage by γ -secretase to release 40 or 42 amino acid long A β fragments. Cleavage of CTF
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14 by γ -secretase releases the APP intracellular domain (AICD) into the cytoplasm.
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22 **Fig. 2.** Proposed model of A β -induced synaptotoxic effects and synapse elimination in AD.
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24 A β is released from vesicles in the presynaptic terminal in an activity-dependent manner.
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26 Increased release or defective clearance results in A β oligomerization. A β oligomers activate
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28 postsynaptic intracellular pathways through a putative A β receptor, leading to AMPA and
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30 NMDA receptor internalization. The postsynaptically silent synapse is a prerequisite for the
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32 subsequent retraction of the presynaptic terminal and the postsynaptic spine, eventually
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34 leading to elimination of the synapse.
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Table 1. Typical T-tau, P-tau and A β 42 test results in relation to control levels in Alzheimer's disease and important differential diagnoses.*

| Diagnosis | CSF biomarkers | | |
|--------------------------------|------------------------------|-------------------------|-----------------------------|
| | T-tau | P-tau | A β 42 |
| AD | Increase | Increase | Decrease |
| Normal aging | Normal | Normal | Normal |
| Depression | Normal | Normal | Normal |
| Lyme disease | Normal to mild increase | Normal | Normal to mild decrease |
| B-vitamin deficiency | Normal | Normal | Normal |
| Neuroinflammation | Normal | Normal | Normal to mild decrease |
| Alcohol dementia | Normal | Normal | Normal |
| PD | Normal | Normal | Normal |
| PSP | Normal | Normal | Normal |
| CJD | Very marked increase | Normal | Normal to marked decrease |
| FTD | Normal to mild increase | Normal | Normal to moderate decrease |
| LBD | Normal to mild increase | Normal to mild increase | Moderate decrease |
| VaD | Normal to mild increase | Normal | Mild to moderate decrease |
| Acute stroke | Mild to very marked increase | Normal | Normal |
| Non-acute CVD without dementia | Normal | Not examined | Normal |

1 Abbreviations: AD = Alzheimer's disease; PD = Parkinson's disease; PSP = progressive
2 supranuclear palsy; CJD = Creutzfeldt-Jakob disease; FTD = frontotemporal dementia; LBD
3 = Lewy body dementia; VaD = vascular dementia; CVD = cerebrovascular disease.
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6 *Absolute concentrations may vary between different studies and laboratories making it
7 important to evaluate biomarker data in relation to the local laboratory's own reference limits
8 or cut-off values (Buerger et al., 2009; Verwey et al., 2009).
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Figure 1

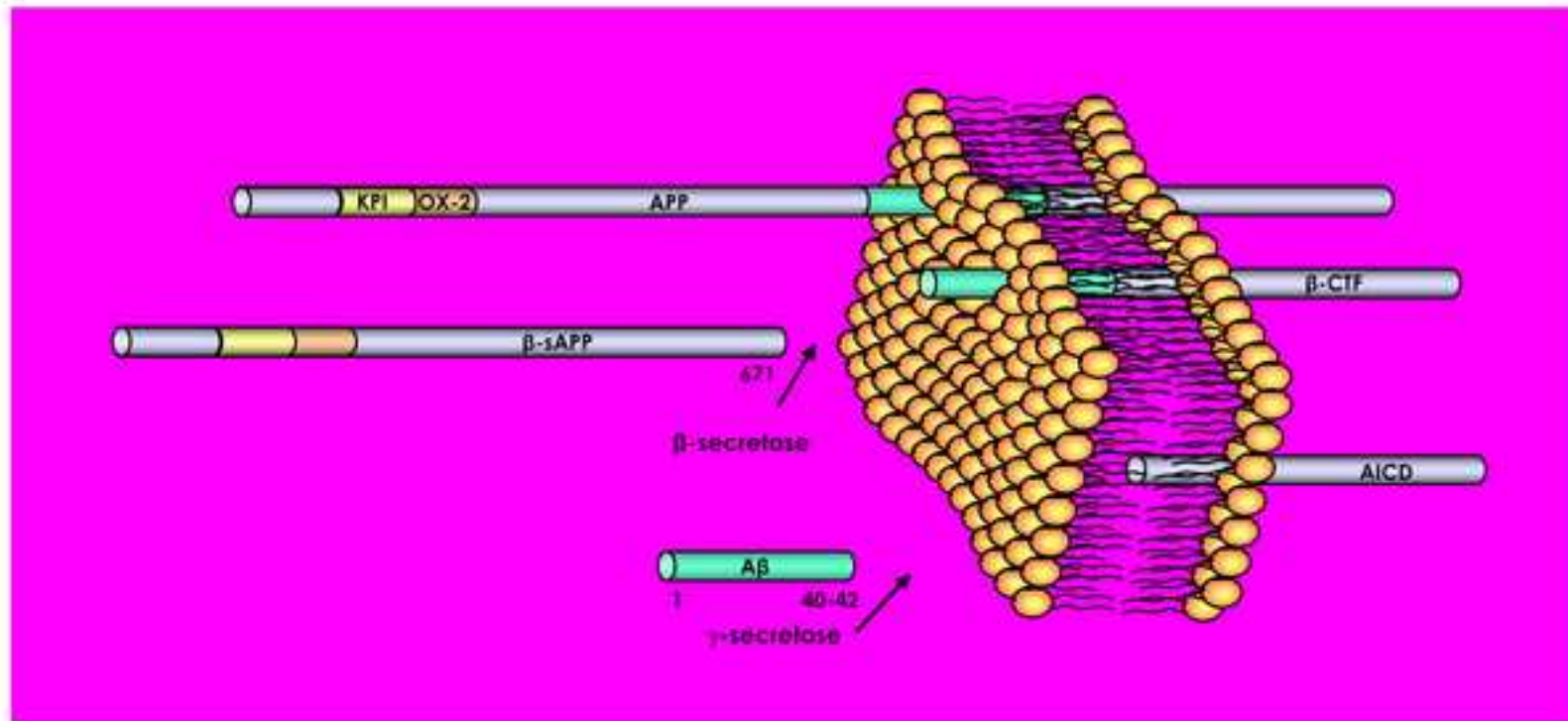
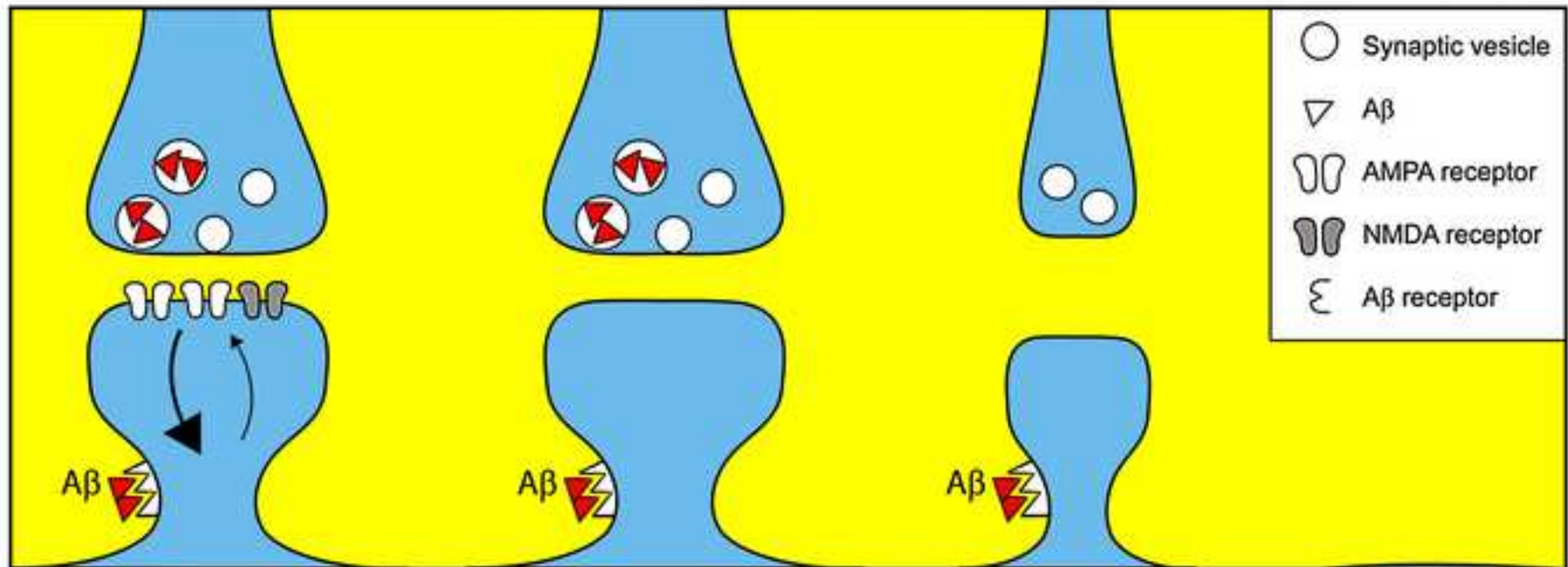


Figure 2



- Inhibition of LTP
- ↑ LTD
- ↑ Removal of glutamate receptors



Postsynaptically silent synapse



- Spine retraction
- Dismounting of presynaptic terminal



Synapse elimination